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Full Length Research Paper

Maximizing seed production during segregating generations of crosses, germplasm maintenance and hybridization processes for breeding

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Efficacy of pollination bags made of new nonwoven fabrics was compared with the traditional paper bags in sorghum during 2015 using three cultivars comprising BR007B (red seeded), SC283 (white seeded) and 1167048 hybrid with tannin (brown seeded). The five pollination bag treatments were: no bagging, traditional paper bag, paper bag plus plastic screen bag for extra bird protection, duraweb$^{®}$ SG2 polypropylene bag and duraweb$^{®}$ SG1 polyester bag. There was no bird damage on tannin hybrid but birds damaged bags to access grains of the other two varieties. Varieties and bag types differed significantly, and also showed significant interactions for panicle weight (at *P*<0.06), seed weight and average seed weight per panicle. The tannin hybrid was consistently a better performer for all traits regardless of bag type. The paper bags were the worst for bird damage. Duraweb® SG1 was the best performer for all traits including bird damage followed by duraweb$^{®}$ SG2. The joint regression analysis showed that BR007B performed consistently under all bag types with average response. On the other hand, SC283 improved its response with the increasing quality of bag type at an above average rate for panicle weight and seed traits. It was concluded that new nonwoven fabric bags could replace paper bags in providing better seed production potential and greater protection against bird damage.

Key words: Sorghum, pollination bags, panicle weight, seed weight, bird control.

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) has great inherent variation with as many as 40,000 germplasm accessions in the US sorghum collection alone, in addition to germplasm collections of many countries of their own (Dahlberg et al., 2011). Maintenance of these germplasm accessions and breeding lines at numerous research stations is facilitated by isolating the genetic accessions and breeding lines from contamination with foreign pollen. This is achieved by the use of pollination control bags. Pollination bags are not only used in artificial hybridization or self-pollination but also for controlling bird damage in the extremely small plots of thousands of germplasms accessions and breeding lines (Ormerod and Watkinson, 2000; Gitz et al., 2013, 2015). Traditionally, plant breeders have been covering the panicles of sorghum with paper bags for pollen control and to protect developing seeds from bird damage and for hybridization of different types of sorghum for genetic

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improvement. Such bags are not very effective against bird damage because the birds over time associate the paper bags, which they can tear off with their beaks, with food of developing seeds underneath. Also paper bags get torn off in the rainy season and heavy winds during the hybridization process. This may lead to high losses of the valuable hybrid seed in the breeding process. Gitz et al. (2013) tested the efficacy of Tyvek® polyethylene bags and found them resistant to bird damage. Gitz et al. (2015) compared the polyethylene and paper bags for pollen transmission and microenvironment within them as this affects seed development. They reported no pollen transmission differences between hard form Tyvek® polyethylene and paper bags but the soft form Tyvek® polyethylene bags allowed 35 to 40% wind borne pollen through the pores. However, heating within the soft and hard polyethylene bags was 25 and 50% that of paper bags, respectively. These studies clearly indicated the need for studies on alternatives to commonly used paper bags in sorghum.

An enormous variety of synthetic fabrics can be made with both woven and nonwoven techniques, and by using knowledge of the polymers, manufacturing processes and fiber properties it is possible to identify fabrics which may produce near-ambient micro-environment within pollination bags for seed development. However, plant breeders have not paid much attention to pollination bags and limited studies have been conducted to compare their efficacy particularly in sorghum (Gitz et al. 2013; Gitz et al. 2015). Gitz et al. (2013) while looking for solutions for maximizing seed yield of breeding/germplasm lines for mechanical sowings by minimizing bird damage could not find off the shelf pollination bags and were unable to identify bags specifically for sorghum. A few studies on rye grass (Griffiths and Pegler, 1963; Foster, 1968; McAdam et al., 1987), switchgrass (Vogel et al., 2014) and trees (McGranahan et al., 1994; del Rio and Caballero, 1999; Neal and Anderson, 2004) highlight the importance of choosing the most efficient pollination bags. PBS International has developed a nonwoven material, duraweb®, specifically for plant breeding purposes, although the researchers believed that this particular material could be developed further for the purposes of this application to increase airflow.

The objectives of this study were to compare the efficacy of two novel materials identified and developed by PBS International for the purpose of experimentation in sorghum against traditional paper bags and to evaluate the effect of different bag types on the performance of different varieties for some seed harvest traits. Such traits include their relative protection against bird damage. The overarching aim was to maximize seed production during segregating generations of crosses, germplasm maintenance and hybridization processes for breeding purposes.

MATERIALS AND METHODS

The present investigation was carried at the Embrapa Milho e Sorgo in Sete Lagoas, MG Brazil research station during 2015. The experiment was conducted during the winter season in a split-plot design with three varieties in the main plots and five bag types in the sub-plots. There were four complete replicate blocks in the experiment. Each sub-plot consisted of one five meter row with 70 cm spacing between rows having 8 to 10 plants per meter.

Three varieties were distinct for the seed coat colour. This was purposely done to see if there is any relationship of seed coat color and bird choice. The varieties were: BR007B with red seeds; SC283 with white seeds, and 1167048 – a brown seeded experimental hybrid with tannin (bird resistant) and referred to as Tannin line hereafter. Panicles were covered by pollination bags before pollination. There were five bag treatments:

1. No bagging (control)
2. Normal Kraft paper pollination bag
3. Normal Kraft paper pollination bag covered by a plastic screen bag for extra protection following pollination and at seed formation.
4. Duraweb® SG2 pollination bag of size 400 mm x 215 mm made from nonwoven polyester with a smooth paper like surface.
5. Duraweb® SG1 pollination bag of size 400 mm x 215 mm made of coarse nonwoven polypropylene with a point-bonded surface

Of the 5 rows of a variety whole-plot in a replication block, one row was allocated to each of the 5 bag treatments. Five panicles were covered by each pollination bags in a row of a variety plot. Observations were made on all 5 panicles in each plot. Data were collected on number of panicles per treatment, panicle weight (g) and average seed weight (g) per panicle. Each panicle was threshed separately in a head thresher and seed weight was recorded in grams. There was slight variation in the panicle number per treatment. Therefore, we performed a covariance analysis using panicle number as the covariate following Snedecor and Cochran (1974) for all traits and using MINITAB 16 package. When the covariance with panicle number was not significant then the analysis of variance was re-performed without the covariate.

The analysis of varieties x pollen control treatment interactions was performed by fitting linear regressions of variety mean values on to the mean values of each bag type following Yates and Cochran (1938), Finlay and Wilkinson (1963), Eberhart and Russell (1966) and Perkins and Jinks (1968). The mean of bag type equates to environmental indices in these studies. A joint regression analysis was used to characterize the sensitivity (inversely instability) of varieties due to bag effects by partitioning the variety x bag type interaction into heterogeneity of regressions and residual interactions. Since regression of panicle weight was significant on panicle number in the covariance analysis adjusted mean values were used for the joint regression analysis for panicle weight.

RESULTS

Bird damage

It was observed that bag treatments 3 (Paper bag+

Figure 1. Tearing of paper bag by the pushing panicle (left) and no tearing effect on duraweb® SG2 bags.

plastic screen bag at grain filling), 4 (duraweb® SG2) and 5 (duraweb® SG1) were similar and more effective in protecting against birds and insects. Bird damage under no bagging treatment 1 (control) and paper bag treatment 2 was high on white and red seeded varieties. However, no bird damage was observed on the brown seeded hybrid with tannin. The astringency from the tannins is what causes the dry and „pucker‟ feeling in the mouth following the consumption of unripe seed (McGee, 2004).

Tannin is a polyphenolic biomolecule that binds to proteins and various other organic compounds including amino acids and alkaloids. The tannin compounds are found in many species of plants where they play a role in protection from predation, and perhaps as pesticides, and in plant growth regulation (Katie and Thorington, 2006). This deters birds unless there is no other food source available.

The bird pressure in the 2015 winter season was medium as the above average rainfall provided alternative food sources for the birds. No bird damage was observed on the tannin variety. The birds preferred the white and red varieties which appeared equally appealing. It was estimated that about 50% of the panicles were damaged in the uncovered treatment (treatment 1) and in the kraft paper bag condition (treatment 2) about 20 to 25% bags were damaged.

From images taken in the experimental field, we observed that paper bags suffered damage made by the birds and by the growth of the panicles bursting the end of the bag indicating their weakness in protection. We have experienced that in some years, when the bird pressure is particularly high, as much as 100% of paper bags are torn open and the plastic screen bags can even be removed by birds requiring multiple visits to re-enforce them. In contrast, the experimental treatments 3, 4 and 5 in the year of this research did not suffer any damage due to the strength of the materials (Figures 1, 2 and 3). It shows that new bags 4 and 5 have strength similar to a paper bag plus protective plastic screen.

Covariance analysis

Since panicle number was variable across treatments it was introduced as covariate in the analysis of variance for panicle weight, seed weight and average seed weight per panicle. The covariance of panicle number was significant for panicle weight (g) but was non-significant for seed weight and average seed weight per panicle (Table 1). Therefore, analysis for panicle weight reported here is adjusted for the significant regression of panicle weight on variation in panicle number. The covariance takes 1 *df* from the error *df* for panicle weight which are 1 less than that for the other two traits (Table 1).

Analysis of variance and mean performance

There were significant differences between varieties and bag types for all traits. The varieties showed significant

Figure 2. Bird damage holes on paper bag (left) and no damage on duraweb® SG1 bags.

Figure 3. Extent of bird attack with small black birds sitting on panicles of their preferred varieties of white and red seed coats. No such bird attack occurred on brown seeded hybrid with tannin in the seed coat.

interaction with bag types for seed weight and average seed weight per panicle at $P < 0.01$ (Table 1). There was also a near significant interaction between main effects for panicle weight at $P = 0.06$.

Variety mean yield showed that the hybrid with tannin was superior for all three traits. Varieties SC283 and BR2007B were statistical similar for all traits although they changed ranks for seed weight and average seed weight compared to panicle weight. SC283 with higher mean panicle weight showed lower mean values for seed weight and average seed weight than BR007B (Table 2).

Mean performance of bag types showed that bag type 5 (duraweb® SG1) was superior to all other bags (Table 2, Figure 4) for panicle weight and average seed weight. It was followed by bag 4 (duraweb® SG2) which was superior to all for seed weight. Bag 3 (paper with screen)

Table 1. Mean squares from analysis of variance for panicle weight (g), seed weight (g) and average seed weight per panicle (g).

Source	*df*	Panicle weight (g)	Seed weight (g)	Av. seed weight per panicle
Panicle number	1	5431**	NS	NS
Rep	3	1282	606	19.79
Variety	2	131796**	103808**	4731.58**
Error (a)	6	754	442	26.63
Bag treatments	4	5137**	4987**	219.69**
Variety *Treatments	8	1353+	1438**	59.02**
Error (b)	35 (36)	646	424	14.92
Total	59	-	-	-

***P*<0.01; +*P*=0.06; NS =Not-significant. Error (b) *df* in bracket are without covariate analysis for panicle number for seed weight and average seed weight where covariance with panicle number was non-significant.

Table 2. Mean performance of bag types over three varieties and varieties over five bag types for different traits. Grouping was carried out using Tukey Method at 95% confidence for all traits.

Bag type	Panicle wt (g)	Seed wt (g)	Av seed wt (g)	Variety	Panicle wt (g)	Seed wt (g)	Av seed wt (g)
5	162.10^{A}	106.26^{A}	23.34^{A}	Tannin	234.78^{A}	175.41^{A}	37.11^{A}
4	153.52^{AB}	108.62^{A}	22.37^{A}	SC283	98.27^{B}	48.56^{B}	9.92^{B}
3	149.69^{AB}	103.26^{AB}	20.86^{AB}	BR007B	90.26^{B}	52.80^{B}	11.06^{B}
2	129.38^{BC}	82.11^{BC}	17.38^{BC}	LSD 5%	16.32	13.22	2.48
1	110.82^{C}	61.04^{C}	12.87^{C}	LSD 1%	21.89	17.73	3.33
LSD 5%	21.06	17.06	3.20	-	-	-	-
LSD 1%	28.26	22.90	4.30	-	-	-	-

Mean values followed by the same letter do not differ significantly. Bag types; 1= no bagging, 2= paper bag, 3= paper bag + plastic bag, 4= duraweb ® SG2, 5= duraweb® SG1. LSD = least significant difference.

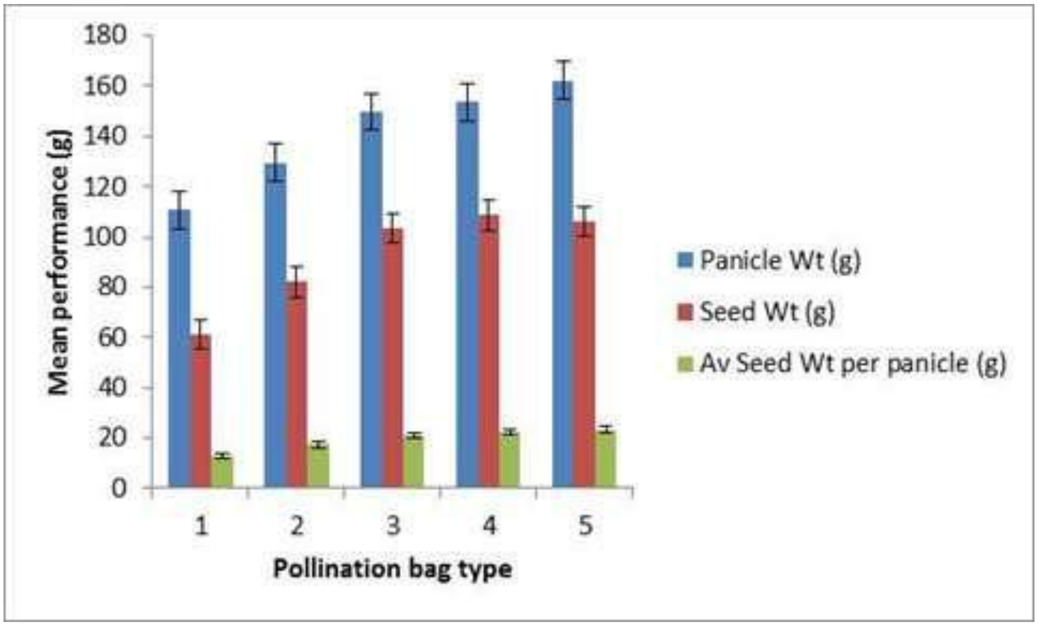

Figure 4. Mean performance of bag types over three varieties for different traits.

Table 3. Variety x pollination bag types interactions for panicle weight (g) after allowing for its covariance with number of panicles, seed weight (g) and average seed weight per panicle (g).

	Bag type[ξ]						
Variety	**1**	**2**	**3**	**4**	**5**	**Var Mean**	**LSD 5% Var Mean**
Panicle weight							
BR007B	32.57	94.56	113.34	100.70	110.12	90.26	16.32
SC283	64.14	79.76	110.20	112.77	124.47	98.27	-
Tannin	235.75	213.82	225.54	247.08	251.69	234.78	-
Bag Mean	110.82	129.38	149.69	153.52	162.10	141.10	-
LSD 5% Bag mean	21.06	-	-	-	-	-	-
LSD 5% Interactions	36.48	-	-	-	-	-	-
Seed weight (g)							
BR007B	0.53	60.06	74.44	71.09	57.88	52.80	13.22
SC283	5.35	27.6	63.15	69.01	77.71	48.56	-
Tannin	177.25	158.66	172.19	185.78	183.18	175.41	-
Bag Mean	61.04	82.11	103.26	108.62	106.26	92.26	-
LSD 5% Bag mean	17.06	-	-	-	-	-	-
LSD 5% Interactions	29.56	-	-	-	-	-	-
Average seed weight per panicle							
BR007B	0.11	12.01	15.51	14.22	13.45	11.06	2.48
SC283	1.14	6.47	12.63	13.80	15.54	9.92	-
Tannin	37.35	33.66	34.44	39.09	41.02	37.11	-
Bag Mean	12.87	17.38	20.86	22.37	23.34	19.36	-
LSD 5% Bag mean	3.20	-	-	-	-	-	-
LSD 5% Interactions	5.44	-	-	-	-	-	-

[ξ] 1= no bagging, 2= paper bag, 3= paper bag + plastic bag, 4= durawed ® SG2, 5= duraweb® SG1.

was inferior to 4, and 5 for seed weight and average seed weight but was similar to bag 2 (paper bag). Statistically, Bags 4, 5 and 3 fall in the same group for all traits. Treatments 1 (no bag) and 2 (paper bag) were similar and inferior for all traits.

Variety x bag type interaction

Variety x pollination bag type interaction was significant for all traits (Tables 1 and 3, Figures 5, 6 and 7). The hybrid with tannin showed consistently higher mean values with all bag types though the magnitude varied over bag types. Thus the hybrid with tannin was least interactive with bag type and performed well with any type of bag. The other two varieties showed a change of ranking resulting in cross-over interactions (Tables 1 and 3, Figure 5, 6 and 7). For instance, for panicle weight Tannin and SC283 varieties showed highest mean values with bag type 5 but BR007B variety with bag type 3. The lowest panicle weight for Tannin was with bag 2 but with bag 1 for the other two varieties. Similarly, rank changes are noticeable for seed weight and average seed weight.

Correlations of bag type with mean values for all traits were positive and significant (Table 4) showing that as the mean performance of the bag type improves from bag type 1 to 5 so does the mean performance for all traits. Mean values of all three agronomic traits were highly correlated over the five bag types (Table 4). How the three varieties performed under different bag types was indicated from their separate correlations for the five bag types.

Variety SC283 consistently showed highly significant correlation with bag type for all three traits showing that its mean performance was associated with improvement in bag type and that it produced better performance under better bag type. This variety is most sensitive to bag change and hence bags for SC283 need to be carefully chosen. For the other two varieties all correlations were non-significant showing that varietal performance for any of the traits was independent of bag type and that any bag type will be equally effective. However, for BR007B the trend for average seed weight was close to significance level and perhaps could be significant if there were more than five bag types providing more degrees of freedom. Thus, variety

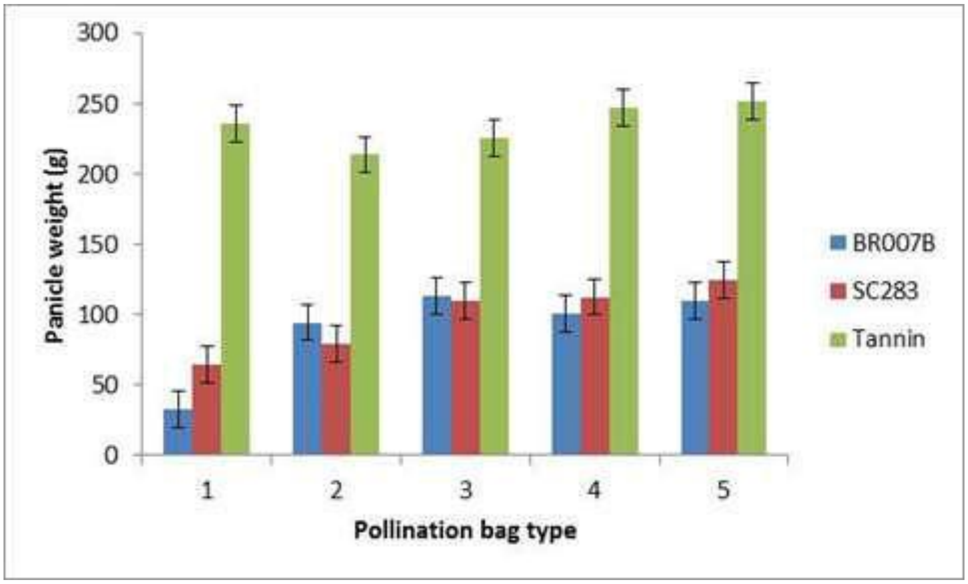

Figure 5. Mean performance of each variety for panicle weight (g) against different pollination bag types.

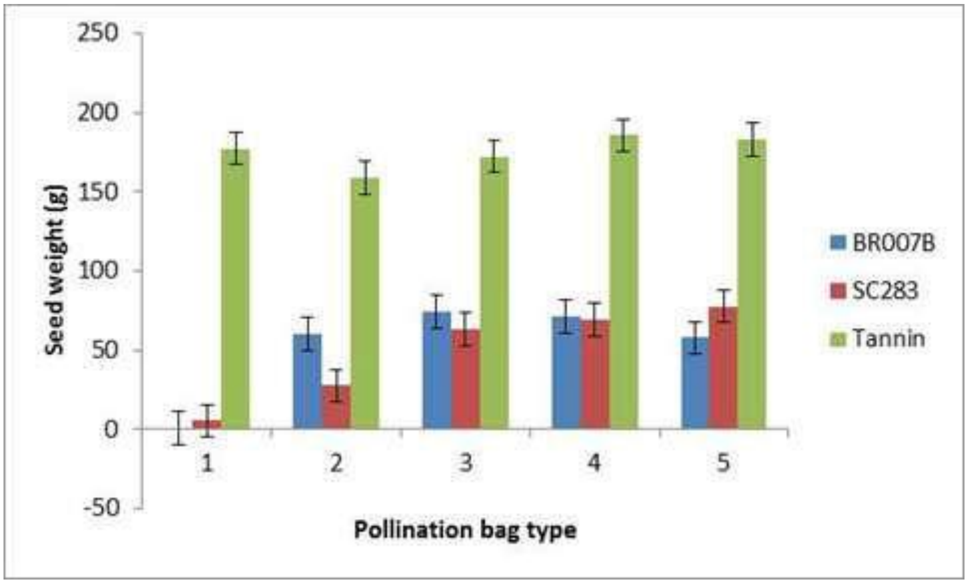

Figure 6. Mean performance of each variety for seed weight (g) against different pollination bag types.

BR007B also has the tendency to show inter-relationship with bag type.

Joint regression analysis

A joint regression analysis of individual variety means on to the all variety means for each bag type was performed for all traits (Table 5). For panicle weight mean values over four replications adjusted for covariance with panicle number were used. The significant heterogeneity among regressions for all traits showed that linear interactions were important (Table 5). However, for average seed weight the significant remainder mean squares indicated the importance of both linear and non-linear interactions (Tables 5).

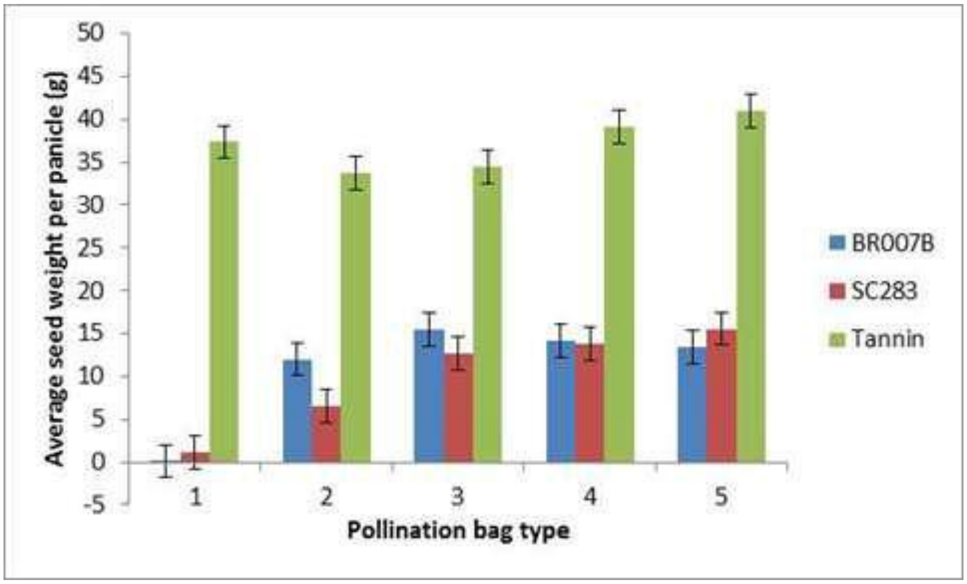

Figure 7. Mean performance of each variety for average seed weight per panicle (g) against different pollination bag types.

Table 4. Correlations between mean performance of agronomic traits over bag types and with five bag types.

Variable	Bag type	Panicle Wt (g)	Seed Wt (g)	Variety	Panicle wt Vs bag type	Seed Wt Vs bag type	Av seed Wt Vs bag type
Panicle Wt (g)	0.965**	-	-	BR007B	0.770	0.661	0.839
Seed Wt (g)	0.907*	0.978**	-	SC283	0.963**	0.956**	0.971**
Av Seed Wt (g)	0.958*	0.996**	0.987**	Tannin	0.664	0.573	0.305

**P*<0.05; ***P*<0.001. Table value of *r* at 3 *df* = 0.878 at 5% and 0.959 at 1% levels.

Table 5. Joint regression analysis (mean squares-MS) of varieties on to the mean of all varieties for a bag type for panicle weight (g) on means adjusted for the covariance with number of panicles, seed weight and average seed weight per panicle (the analysis was based on means over four replications).

Source	*df*	MS for panicle weight (g)	MS for seed weight (g)	MS for Av seed weight (g)
Variety	2	32987**	25952**	1183**
Bag types	4	1292**	1247**	55**
Variety x Bag types	8	340+	360**	15**
Heterogeneity of regressions	2	671*	845**	31**
Remainder	6	230	198	9*
Error	35 (36)	161	106	4

**P*<0.05; ***P*<0.01; +*P* = 0.05-0.10. Error *df* in bracket are for seed weight and average seed weight without adjustment for covariance for panicle number.

The Tannin cultivar clearly showed higher productivity with highest panicle weight, seed weight and average seed weight per panicle with all type of bags; this variety did not show any dependency on bag types and all types of bags were equally suitable for this variety. Apparently, Tannin hybrid did not have a significant regression on bag types for any trait (Table 6 and Figure 8).

Trends for other two varieties were similar for all traits

Table 6. Estimates of regression parameters for varieties on to mean of all varieties under different pollination bag types for panicle weight (g), seed weight (g) and average seed weight per panicle (g).

Variety	Panicle wt (g)	Seed wt (g)	Av seed wt per panicle (g)
BR007B	-109.67+1.42±0.42*,ns; R^2=79%	-67.44+1.30±0.40**,ns; R^2=78%	-14.05+1.30±0.39**,ns; R^2=79%
SC283	-72.31+1.21±0.07**,**; R^2=99%	-88.24+1.48±0.16**,**; R^2=97%	-17.04+1.39±0.07**,**; R^2=99%
Tannin	181.98+0.37±0.37ns; R^2=25%	155.68+0.21±0.28ns; R^2=17%	31.09+0.31±0.38ns; R^2=19%

ns= non-significant; *P<0.05; **P<0.01. The first significance for regression coefficients is from zero and the second is from 1.0.

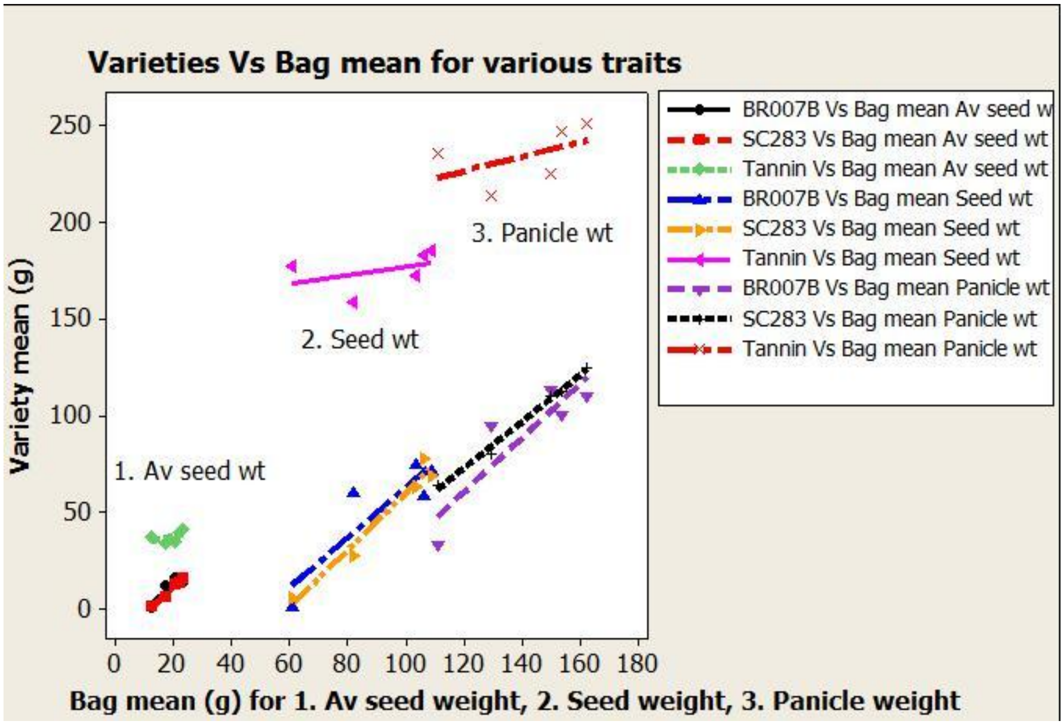

Figure 8. Scatter plots for regression of mean performance of varieties for 1. Average seed weight (g), 2. Seed weight (g), and 3. Panicle weight (g) on to mean of all varieties for each pollination bag type. For estimates of regression parameters see Table 6.

(Table 6 and Figure 8). Variety BR007B showed an average regression of unity for all traits. Hence this variety responds generally well to all bag types showing an average increase in performance with the improvement of bag type.

However, variety SC283 shows an above average response of greater than unity for all traits (Table 6 and Figure 8) which means its performance increases at above average level in response to the improvement of bag type‟s performance as was also shown by significant correlation coefficients in Table 4. Consequently, it is specifically suitable for bags with higher performance, that is, duraweb® SG2 and duraweb® SG1 (bags 4 and 5). It may be noted that as the bag type improves the difference in performance of BR007B and SC283 gets reduced due to higher rate of response of SC283.

DISCUSSION

The primary function of pollination bags is to facilitate the process of pollen control and hybridization between potential parents. Bagging of plants creates a fabric barrier between reproductive parts and the environment, and is practised to control pollen transmission by insects, wind or other agents and also to collect pollen for artificial cross pollination. Another use of bagging is to facilitate self-pollination of plants and to protect against bird damage to developing grains particularly in very valuable materials in the breeding nursery including inbred lines and germplasms.

A plant breeder always aims at maximising the seed production under controlled breeding for experimentation while minimising the seed loss from bird attack, insects or damage from environmental vagaries by protecting with pollination bags. While paper bags are commonly used in sorghum other types of materials made from muslin, micromesh, polyethylene, cellulose acetate, micropore acetate bread bags have been in vogue in other plant breeding researches on various types of plants (Pickering, 1982; Ball et al.,1992; Wyatt et al., 1992; McGranahan et al., 1994; del Rio and Caballero, 1999; Neal and Anderson, 2004; Gitz et al., 2013, 2015).

New synthetic materials have been developed which have greater strength for bird owitchgrassr wind resistance, more air permeability, less moisture absorption and resistance against pollen contamination (PBS International, 2013). Polyester bags have been successfully used to control pollination in tree species such as *Elaeis guineensis*, *Melaleuca alternifolia*, *Grevillea robusta* and *Phillyrea angustifolia* (PBS International, 2016). Although materials used for bagging plants have specific merits and demerits the sorghum breeders have not changed over to any other materials than brown paper bags perhaps because of their low cost, availability or adherence to age-old practice.

It is important that studies on performance of paper bags and new fabrics are conducted to build the confidence of sorghum breeders to try new options of nonwoven materials for bagging plants in the breeding processes. Studies have shown that different materials vary in permeability and pollen proofing (McAdam et al. 1987; Adhikari et al., 2014; Vogel et al., 2014). Vogel et al. (2014) obtained four to tenfold increase in seed produced per cross in micro-mesh fabric pollination bags in switchgrass that allowed larger progeny for evaluation in replicated trials. Adhikari et al. (2014) reported that polyester bags were more reliable than traditionally used bags in controlling contamination by foreign pollen using simple sequence repeats (SSR) markers to identify extent of contamination by outcrossing in the bagged panicles of switchgrass for selfing of progenies.

The micro-environment within pollination bags can vary greatly depending upon the type of fabric. Therefore, identifying fabrics that create appropriate environmental conditions within the bag is crucial (Foster, 1968). Gitz et al. (2015) compared the microenvironments within novel spun-bond polyethylene and brown paper bags in sorghum. A considerable increase in temperature was measured within brown bags throughout the season as compared to ambient temperatures.

However, temperatures within polyethylene bags were lower than paper bags because of air permeability. Humidity was lower in soft polyethylene bags than hard polyethylene and paper bags that resulted in moulds especially in the recently irrigated plants. Hayes and Virk (2016) found in *Miscanthus* that duraweb$^{®}$ bags exhibited a narrower range of temperature and humidity than those shown by the Orchard and Glassine bags which could impact the success of crossing and seed set rate. The duraweb$^{®}$ bags made from nonwoven polyester seem to allow air-permeability and moisture absorption for micro-environmental adjustments conducive for better seed set and development.

Bird attack is a major problem in sorghum breeding and germplasm maintenance. Paper pollination bags are damaged by rains and provide minimal deterrent to birds (Gitz et al., 2013). The study results show that covering of panicles with synthetic nonwoven bags provides protection against birds and the damage and seed loss by birds was nearly eliminated under the novel bags. This observation is specifically relevant to areas where bird damage on sorghum breeding stocks is serious. This also is relevant to areas with unpredictable climatic conditions.

Plant breeding experiments often have differential plant stand especially in dry and rainfed conditions due to uneven seedling survival. Trabanino et al. (1989) reported that sorghum seedling stands in Central Honduras are influenced by the infestation by ants, white grubs and armyworms. In the event of variable plant stand resulting from any causes an analysis of covariance that combines the features of analysis of variance and regression is highly useful in computing adjusted means (Snedecor and Cochran, 1974). The study found that the total panicle weight was influenced by the variation in panicle number but seed weight and average seed weight were not affected by the variation in panicle number. Thus adjustments by covariate analysis were justified.

The study showed that paper bags were consistently inferior in performance whether for resistance against bird damage or for panicle and seed traits irrespective of the variety. The new bags, on the other hand, produced more panicle weight, seed weight and average seed weight perhaps due to better micro-environments within them as reported by Hayes and Virk (2016) in *Miscanthus* and Gitz et al. (2015) in sorghum. There were significant interactions of varieties with bag types for seed weight and average seed weight. Variety Tannin did not show significant interaction with bag types and hence its performance for various traits did not depend on bag types. This variety was the highest performer for all traits and showed no bird preference.

While SC283 showed greater than unity regression with above average response to bag types compared with BR007B that showed an average response to changes in bag types showing that its performance improves at the rate of improvement in bag type performance.

Table 7. Factors for comparing pollination bags for economic analysis.

Treatment$^{\xi}$	Bird damage observed	Relative bag cost	Other cost implications	Risk of catastrophic loss	Reusability
2	20-25%, up to 100% in high pressure seasons	$	Extra bags, labour to check / replace bags; Over-planting to compensate for loss	Yes, under high pressure	Not reusable
3	0% in current study. 20% observed in high pressure years	$ $	Extra labour cost to attach screens	Some risk under high pressure; damage and lower seed yield	Screen bag reusable
4	0% (not tested under high pressure)	$ $	No extra labour in normal year	Little (not tested under high pressure)	Highly probable† (not tested here)
5	0% (not tested under high pressure)	$ $	No extra labour in normal year	Little (not tested under high pressure)	Highly probable† (not tested here)

$^{\xi}$ 2=paper bag, 3= paper bag + plastic screen bag, 4 =duraweb® SG2, 5= duraweb® SG1. † Hayes and Virk (2016) found duraweb® bags reusable in *Miscanthus*.

It means that better performing bags will be comparatively more useful for all varieties that are more prone to bird attack and that higher specification bags may be required for some varieties such as SC283. Clearly, more research needs to be conducted before generalisations are made about different bag types but what is clear is that the novel bags performed better than the traditional practice of paper bags in all circumstances within our experimentation.

Economic analysis

While these studies do not support a proper economic analysis to compare various bag types we can examine essential factors that determine their comparative advantages as a preliminary attempt. We have set out a scenario in Table 7. It should be emphasised that pollination bags have more relevance in the breeding processes than in commercial seed production.

During the filial generations, seed produced is always in small quantities from individual lines or plants and if such progeny are lost due to bird damage then the whole year is virtually wasted at the loss of labour and effort used in the season. The necessity of protection against bird damage becomes more severe when there is high bird pressure especially in the off-seasons where alternative sources of food are scarce. We have noticed that under the medium pressure as, in the present study, the mean seed weight of variety BR007B under no bagging was only 1% of the overall mean performance under all treatments tendering a loss of 99% (Table 3).

Similarly, variety SC283 showed only 11% performance of mean registering a loss of 89%. However, there was no loss in the Tannin variety. Thus on average, 90% seed weight is lost in bird susceptible varieties which can be avoided by putting bags 3, 4 or 5. On average, new bag types 4 and 5 produced 32 and 29% more seed weight than the paper bags on the basis of mean over all varieties (Table 3). This is a significant economic benefit from the novel bags even under medium bird pressure in the present experiment.

To allow for light or moderate bird pressure, excess resources such as labour, seed, land and consumables have to be used to ensure that the target seed yield is achieved. For example, if 25% seed loss is typical, 33% more seeds should be sown to allow for bird related reduction. In addition, extra labour is required to patrol the fields and replace damaged bags as and when required.

However, in some years the bird pressure is severe and up to 100% seed loss results when paper bags are used. Under these circumstances the entire direct cost of the programme (which may be as high as several hundred thousand dollars) is wasted, and an entire breeding cycle is lost, delaying the progress of the work. Although the new materials have not been tested under these circumstances, it is thought that the protection against bird damage may reduce the risk of catastrophic loss of this type. In addition to this the researchers felt anecdotally that the seeds produced under the paper bags were of lower quality, a topic for future research.

Finally, the nonwoven bags are likely to be re-useable, thus reducing the cost-per-cross of the bags, although this was not tested in this experiment. These preliminary results need confirmation with more robust experiments to explore the economic implications more fully, and to establish whether micro-environmental differences in the bags explain differences in their seed harvest outcome.

Conclusion

The use of carefully selected nonwoven bags instead of commonly used paper bags for germplasm maintenance and crossing purposes is recommended, since these bags provide better protection against bird damage as well as higher panicle weight, seed weight and average seed weight per panicle across all three types of varieties of sorghum.

Conflict of Interests

The authors have not declared any conflict of interests.

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